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***Review:***

**Novel anti-angiogenic therapeutic strategies in colorectal cancer**

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**Abstract**

***Introduction:*** Anti-angiogenetic agents are currently the standard of care in metastatic CRC patients. Bevacizumab, aflibercept, regorafenib and recently ramucirumab have significantly improved both progression-free and overall survival in different lines of treatment. Since bevacizumab's approval, a number of novel anti-VEGF agents have been tested in preclinical and clinical models.

***Areas covered:*** This review is focused on the most recent clinical results of novel agents targeting VEGF and its receptors with a major focus on those investigated recently in clinical trials.

***Expert opinion:*** In the last 15 years, a number of new anti-angiogenetic agents have been tested. Unfortunately, most of them have demonstrated unacceptable toxicities or failed to show activity. When tested as single agents, encouraging preliminary results were reported with fruquintinib,

famitinib, and nintedanib. Interesting novel mechanisms of action are also being explored: VGX-100 is a monoclonal antibody (mAb) which binds to VEGF-C, inhibiting activation of VEGFR-2 and VEGFR-3 when combined with bevacizumab; tanibirumab is a mAb which binds to VEGFR-2 and vanucizumab is a bispecific mAb binding both to VEGF-A and Angiopoietin-2. Data about the combination of these agents with chemotherapy are very encouraging, even though preliminary. However, the definition of specific predictive biomarkers remains a priority.

**Key words:** angiogenesis, anti-VEGF inhibitors, colorectal cancer, receptor TKI



## ARTICLE HIGHLIGHTS BOX

- Angiogenesis is a complex process, mainly regulated by VEGF pathway, and its inhibition is safe and active in patients with metastatic colorectal cancer (mCRC). Several mechanisms of intrinsic or acquired resistance to anti-VEGF therapy have been hypothesized.
- Several agents have been authorized by regulatory agencies for the treatment of mCRC patients: among these drugs, monoclonal antibodies (bevacizumab and ramucirumab), a recombinant fusion protein consisting of portions from human VEGF receptors 1 and 2 fused to a portion of the human IgG1 immunoglobulin (aflibercept) and an oral multi-kinase inhibitor (regorafenib).
- No patient selection is possible, because no predictive marker of response has ever been described and validated.
- Only few circulating biomarkers, dynamically evaluated during therapy, have shown a potential predictive role, although their clinical utility in this sense has not been demonstrated.
- New anti-angiogenic compounds have been tested, the great majority of them are multi-kinase inhibitors. Unfortunately many of them have been abandoned, due to poor activity or unfavorable safety profile.

## 1. INTRODUCTION

Colorectal Cancer (CRC) is a leading worldwide health care problem. In 2012 it is estimated that more than 1.300.000 new patients have been diagnosed, and more than 690.000 died from this neoplasia [1]. The incidence of CRC has increased in those countries where the risk for this disease was historically low, and remained stable or even declined in high-risk/high-incoming countries. Conversely to incidence trends, decreasing mortality rates have been observed in a large number of countries and are most likely attributed to CRC screening, and/or improved treatments. Specifically, in the last decade new agents and new treatment strategies broadened clinical treatment choices, mainly in the metastatic setting. Besides fluoropyrimidines, oxaliplatin and irinotecan all representing the cornerstone of the systemic treatment of CRC patients, anti-Epidermal Growth Factor Receptor (EGFR) agents and anti-angiogenic agents entered successfully into the clinical practice.

Since 1971, Folkman demonstrated the relationship between neo-angiogenesis and tumor proliferation, providing the basis for a new anti-tumoral strategy [2]. Since then a number of new anti-angiogenic agents have been developed and tested in clinical trials, confirming that targeting the tumour angiogenesis could effectively treat cancer. Unlike traditional chemotherapeutic drugs, angiogenesis inhibitors specifically target the formation of new blood vessels, do not interfere with dividing hematopoietic stem cells or dividing epithelial cells. This results in a treatment-related toxicity profile completely different to that is usually observed with cytotoxic agents. In fact, the main related side effects of bevacizumab are hypertension, proteinuria, thromboembolic events, haemorrhage, and bowel perforation. Toxicities are usually mild, with a limited number of grade 4 or grade 5 episodes [3]. Because the majority of the tumours require neo-angiogenesis, angiogenesis inhibitors have the potential to treat a variety of neoplasms. Most of the tested anti-angiogenetic agents are likely to have limited single-agent activity leading to a cytostatic effect rather than

cytotoxic, and in some cases dose-limiting toxicities may not been seen; indeed, the side effects are often shown. The main limitation of this class of agents is the completely absence of a biomarker of drug activity.

Novel anti angiogenetic agents are emerging. In this review, we focus the attention to these new agents outlining their characteristics, mechanisms of action and preliminary results.

## 2. VEGF PATHWAY AND ITS INHIBITION

In mammalians, the most studied pathway involved in the angiogenesis is the Vascular Endothelial Growth Factor (VEGF) family that consists of five glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D,and placenta growth factor) and three receptors (VEGFR-1, VEGFR-2 and VEGFR-3) (Figure 1). In general, VEGFs are soluble growth factors secreted by tumor cells and stromal cells that mediate their effects through specific binding to three different cell membrane receptors: VEGFR-1 (Flt-1) and VEGFR-2 (Flk/KDR) and VEGFR-3 (Flt-4) (Figure 1) [5]. These receptors consist of an extracellular domain that binds specific VEGF ligands, a trans-membrane domain and an intracellular region that contains a tyrosine kinase domain. VEGFR downstream signalling involves the activation of several pathways including Ras-Raf-MAPK, Scr-FAK, AKT-mTOR, the molecular target of rapamycin. The resulted interaction generates the activation of a number of intracellular signalling cascades promoting endothelial cell survival, proliferation, migration, differentiation and increased vascular permeability (Figure 1).

The antiangiogenic drugs lead to inhibition of new blood vessel growth and vascular regression, vascular normalization, vascular constriction, and have direct effects on tumor cell function. In addition they are offsetting the effects of chemotherapy induction of VEGF levels and the inhibition of VEGF repression of dendritic cell function [6]. Focusing on CRC cells, VEGFR-1 and -2 are expressed and functional in several cell lines, and their activation by VEGF family ligands can activate processes involved in tumor progression and metastasis [7,8]. This finding is coherent with the observed clinical activity of several VEGF-targeted agents in patients bearing CRC.

Tumor angiogenesis can be blocked through several ways. Bevacizumab binds and therefore neutralizes the ligand VEGF-A, preventing its binding and activation of the VEGF receptors. Another ligand-depleting strategy led to the synthesis of aflibercept, a recombinant fusion protein (Aflibercept, Figure 1). The VEGF receptors can also be blocked directly by antibodies to the extracellular domain of the receptor, by small molecules that bind the catalytic kinase domain of the receptor preventing activation of downstream effector signalling molecules (Figure 1). Each of these agents has, therefore, a different pharmacology, different specific target and receptor signalling biology and may have additional and unique properties.

In addition to the VEGF pathway, several other pathways concur to angiogenesis, and mechanism of resistance to anti-VEGF therapy may be due to activation of compensatory angiogenesis factors. Players of this mechanism are soluble angiogenic factors such as placental growth factor (PlGF); the FGF signalling pathway; the Notch ligand and receptor; angiopoietins; recruitment of bone marrow cells that secrete soluble angiogenic factors, increased pericyte coverage of tumour blood vessels to support vasculature; macrophages that also secrete soluble angiogenic factors. Moreover, resistance to anti- VEGF therapy may be due to recruitment of other lymphangiogenic factors, pathways and myeloid cells [9].

## 3. PREDICTIVE MARKERS

Despite rapid advances, a clear strategy to optimize translation of experimental data into clinical practice remains to be defined. Current evidence indicates predictive value for some circulating biomarkers, such as an increase in VEGF or a decrease in circulating endothelial cells. However, so far the clinical usefulness of these and other surrogate biomarkers has yet to be proven [10].

In a phase II trial, Kopetz et al evaluated potential biomarkers of resistance in mCRC patients treated with FOLFIRI plus bevacizumab. Interestingly, they demonstrated that the levels of several other angiogenic factors were modulated by treatment. In particular, circulating levels of PlGF,SDF-1 and MCP-3 (the latter two factors are known to be potent chemoattractants for myeloid cells and hematopoietic progenitor cells) increased during treatment with bevacizumab in progressing patients [11]. The elevation of circulating PlGF levels in patients progressing during bevacizumab containing regimen was further confirmed in a retrospective study enrolling a total of 450 patients [12] and suggested in another study [13].

Many studies investigated the relationship between systemic hypertension induced by anti-VEGF therapy and patient outcome. In some of these studies, in fact, the occurrence of grade 2 or more hypertension during treatment with bevacizumab was correlated to best Overall Response Rate (ORR) and prolonged Progression-Free (PFS) and Overall Survival (OS) [14-17]. Conversely, one study did not find any correlation [18]. The main limitations of all these studies are the relative low number of patients considered and the retrospective nature of the statistical design, characteristics that prevent any definitive conclusion.

## 4. EXISTING THERAPIES

To date, anti-angiogenic agents have not shown efficacy as adjuvant treatment in non-metastatic CRC patients, whereas four of them showed clinical activity in comparative trials and are approved for the clinical management of metastatic patients across different lines of therapy: namely, bevacizumab, aflibercept, regorafenib, and ramucirumab (the latter only in the US market, awaiting for EMA approval). These agents demonstrated to be easily manageable, with an acceptable safety profile. The most frequent grade 3-4 side effects reported with their use are hypertension (10%-15% of patients), thromboembolism (10%), proteinuria (8%), hemorrhage (5%) and bowel perforation. Moreover, the addition of anti-angiogenic agents to chemotherapy increases the adverse event profile of cytotoxic agents compared to chemotherapy alone, increasing the frequency of diarrhea,

fatigue, nausea and/or vomiting, stomatitis, and anorexia [19]. The most studied anti angiogenetic agent is bevacizumab (AVASTIN™, Roche), a humanized monoclonal antibody that binds to VEGF-A and prevents interaction between VEGF-A and VEGFR. Combined to standard chemotherapy, bevacizumab has demonstrated to prolong PFS and OS either in first or in second line treatment [20]. Moreover, patients could benefit from bevacizumab as maintenance therapy following first-line regimen and even beyond progression [21]. Very recently, two meta-analyses and an Italian phase III randomized trial questioned about the best chemotherapy partner to be combined to bevacizumab. In fact, these studies demonstrated no difference in adding bevacizumab to standard FOLFIRI or FOLFOX regimen, being capecitabine or schemes containing bolus 5-fluorouracil those that demonstrated better results [22-24]. Finally, in the adjuvant setting the addition of bevacizumab to the standard FOLFOX6 regimen did not demonstrate to improve disease free survival in two large phase 3 studies conducted in stage II and III CRC patients [25,26] .

Aflibercept (ZALTRAP™, Sanofi) is a fully humanized recombinant soluble fusion protein comprised of segments of the extracellular domains of VEGFR-1 and -2 fused to the constant region (Fc) of human IgG1 with potential antiangiogenic activity. This drug functions as a soluble decoy receptor, binds to pro-angiogenetic VEGFs, and placental growth factors 1 and 2 (PlGF-1 and -2) thereby preventing VEGFs and PlGF from binding to native VEGF receptors, and therefore inhibits angiogenesis. Addition of aflibercept to second-line FOLFIRI prolonged both PFS and OS compared to placebo arm [27]. Furthermore, the overall response rate (RR) of patients treated with aflibercept was superior to placebo group (19.8% vs 11.8%). Today, aflibercept combined to FOLFIRI is indicated in patients progressing after an oxaliplatin-based chemotherapy.

Regorafenib (STIVARGA™, Bayer) is an oral multikinase inhibitors with activity against a range of tyrosine kinases (VEGFR2-3, TIE-2, PDGFR, FGFR, RET and c-Kit) as well as a signal transduction inhibitor of the RAF/MEK/ERK pathway [28]. Regorafenib demonstrated activity versus placebo in CRC patients progressing after standard chemotherapies plus biological targeted agents (anti-VEGF in all cases and anti-EGFR in RAS wild type tumors). A phase III, multicentre,

placebo controlled study (CORRECT) was performed in 760 CRC patients randomized in a 2:1 ratio to receive regorafenib plus best supportive care (BSC) vs placebo plus BSC. Median survival time of patients randomized to regorafenib was longer than placebo group (6.4 vs 5.0 months) [29]. On the basis of this study, regorafenib is currently indicated in patients refractory to standard chemotherapy. The main limiting toxicities are fatigue and hand-foot skin reactions, which needed appropriate treatments [30].

Ramucirumab (CYRAMZA$^{TM}$, Eli Lilly) is a human antibody against VEGFR2, that is considered the main mediator of tumor angiogenesis. In a phase I study ramucirumab was well tolerated with an interesting activity profile [31]. Ramucirumab demonstrated single agent activity in metastatic gastric cancer patients and it is currently indicated in this patient setting alone or in combination with paclitaxel after failure of a first line chemotherapy. More recently, ramucirumab combined to FOLFIRI as second-line treatment in metastatic CRC patients demonstrated to prolong survival versus FOLFIRI alone. In the RAISE study, a multicentre phase III double blind placebo controlled trial [32], in an intention to treat analysis the median survival time was 13.3 vs 11.7 months in the ramucirumab and placebo group, respectively. Grade 3-4 ramucirumab toxicities were neutropenia (38% of the patients), hypertension (11%), diarrhoea (11%), and fatigue (12%). On the basis of this study ramucirumab has been recently approved by the Food and Drug Administration Agency and is currently under review by EMA for authorization in CRC patients.

## 5. NOVEL ANTI ANGIOGENIC AGENTS

A number of novel anti VEGF agents have been developed in the last few years. Unfortunately, some of them demonstrated marginal activity or had an unfavourable safety profile and are nowadays abandoned. Other molecules are continuously arising from screening libraries and preliminary results are presented but definitive validation studies are needed.

## 5.1 Inactive agents or with unfavourable safety profile not currently under development in CRC

### 5.1.1 BRIVANIB

Brivanib (Bristol-Myers Squibb) is an orally available receptor TKI of VEGFR-2 and FGFR1-2 [33]. In animals and in humans, brivanib showed a favourable safety profile and encouraging antitumor activity [34,35].

In a phase I, dose finding study enrolling metastatic CRC patients, brivanib and cetuximab were safely administered at full dose with manageable toxicities and promising PFS benefits [36].

Based on these results a phase III randomized, double blind, placebo controlled trial compared the administration of cetuximab plus brivanib vs cetuximab plus placebo in metastatic patients with K-RAS wild type CRC tumors refractory to previous therapies. Unfortunately the trial did not meet its primary endpoint as OS was not significantly prolonged in brivanib arm despite a significantly advantage in PFS and a favouring trend for better objective response rate. Moreover, the combination regimen was more toxic with higher incidence of grade 3 or more AEs, particularly fatigue, hypertension, rash, gastrointestinal toxicity including abdominal pain, diarrhoea, dehydration, and anorexia [37].

### 5.1.2 CEDIRANIB

Cediranib (AstraZeneca) is a potent TKI that blocks with high affinity all three VEGF receptors, showing some activity against PDGFRβ and KIT. Cediranib demonstrated to inhibit tumor growth in several xenograft models (lung, colorectal, breast, prostate and ovarian cancer) [38].

In a phase I study the activity of cediranib was evaluated in patients with advanced solid tumors refractory to standard therapies. The most frequent histological types were represented by non-small cell lung cancer and colorectal cancer. Oral treatment with cediranib at the dose equal to or inferior to 30 mg/die was well tolerated, the most common Adverse Events (AEs) being fatigue, nausea,

diarrhoea and vomiting. Even though tumors were refractory to prior therapies, cediranib showed some antitumor activity, with a disease control rate of 81% (26 out of 32 patients) [39].

In metastatic CRC patients, the combination of cediranib at the dose of 20 mg or 30 mg with mFOLFOX6 was evaluated in a phase I study which confirmed the manageable toxicity profile of the drug with five out of the nine evaluable patients who achieved a partial response [40].

The activity of cediranib was further explored in a series of phase II and III studies called HORIZON. HORIZON I was a phase II randomized trial aiming to compare PFS between three groups of patients all receiving mFOLFOX6 as second-line treatment plus cediranib 20 mg (group 1), cediranib 30 mg (group 2), or bevacizumab (group 3). No difference in PFS was demonstrated. Patients treated with cediranib 30 mg presented more frequently grade greater than or equal to 3 adverse events, the most common being diarrhoea, fatigue, nausea and hypertension [41]. HORIZON II, a randomized phase III, double blind, placebo- controlled trial investigated the activity of cediranib 20 mg (502 patients) or placebo (358 patients) combined with CAPOX/FOLFOX as first-line treatment. The primary endpoint was met as prolonged PFS was shown in the cediranib arm (8.6 vs 8.3 months, p=0.01); however no benefit in OS, ORR or liver resection rate was shown [42]. The HORIZON III, a phase II/III randomized, double blind trial, compared the activity of the association of mFOLFOX6 with bevacizumab (713 patients) or cediranib 20 mg (709 patients) as first-line treatment. Clinical activity was observed in the cediranib arm as there was no statistically significant difference between treatment arms on the efficacy endpoints examined. However, the predefined boundary for PFS non-inferiority was not met. The cediranib toxicity profile was consistent with previous studies, but led more frequently to chemotherapy delay or discontinuation than bevacizumab [43].

No further studies of cediranib in CRC patients are currently ongoing.

**5.1.3 LENVATINIB**

Lenvatinib (LENVIMA$^{TM}$, Eisai) is an oral multi-targeted receptor TKI that inhibits VEGFR-1, -2, -3, FGFR-1, -2, -3, -4, PDGFRα, c-KIT receptor and RET [44].

Lenvatinib demonstrated a manageable toxicity profile in a phase I study in patients with advanced solid tumour including colorectal cancer, non- small cell lung cancer, sarcoma and other [45]. In preclinical studies, Lenvatinib delayed tumor growth of human CRC xenografts harbouring KRAS mutation by suppressing capillary sprouting through the inhibition of endothelial cell proliferation. Moreover Lenvatinib decreased the density of tumour associated vessels, with the increase of the hypoxic areas within CRC xenografts [46].

Lenvatinib is indicated in patients with differentiated thyroid cancer that can no longer be treated with radioactive iodine and in clinical progression. In the SELECT trial, a randomized phase III study, patients with radioiodine-refractory thyroid cancer were randomized to oral lenvatinib or placebo. Lenvatinib treatment significantly prolonged median PFS compared to placebo [18.3 vs. 3.6 months; hazard ratio (HR) for progression or death 0.21; 95 % CI 0.14–0.31; $p < 0.001$]. ORR was significantly higher in the Lenvatinib group than in the placebo group (64.8 vs. 1.5 %; odds ratio 28.87; 95 % CI 12.46–66.86; $p < 0.001$) [47].

The efficacy of Lenvatinib is under evaluation in several tumour types including unresectable hepatocellular carcinoma (NCT01761266), advanced endometrial cancer (NCT01111461), unresectable stage III or IV melanoma with or without V600E BRAF mutations (NCT01136967), advanced or metastatic non-squamous NSCLC (NCT01529112), unresectable or advanced renal cell carcinoma in combination with everolimus (NCT01136733). Lenvatinib is not currently under evaluation in the CRC setting.

Several adverse events after lenvatinib administration were reported, including hypertension, proteinuria, diarrhoea, fatigue, asthenia, decreased appetite, decreased body weight, nausea , stomatitis, palmar-plantar erythrodysesthesia syndrome, arterial and venous thromboembolic events, renal failure, hepatic failure, gastrointestinal fistula, and QT prolongation [45, 47].

**5.1.4 LINIFANIB**

Linifanib (ABT-869, Abbott) is an orally active, adenosine triphosphate (ATP) competitive receptor TKI that has inhibitory activity against VEGFR-1, -2, -3, and PDGFRβ. In the preclinical data, this molecule showed efficacy in several xenograft tumour models, including breast, colon, and small cell lung carcinomas [48].

Linifanib showed single agent in several tumours including non-small lung cancer, CRC, ovarian cancer, hepatocellular carcinoma, neuroendocrine tumor, renal cell carcinoma and alveolar soft part sarcoma [49]. Very recently, linifanib added to standard platinum-based chemotherapy showed to be safe and active in non-squamous NSCLC patients [50].

A randomized phase II study compared the efficacy and safety of mFOLFOX6 combined with two doses of linifanib (7.5 and 12.5 mg, po, qd) or bevacizumab in patients with metastatic CRC progressing after a first-line treatment. The study did not meet its primary endpoint, as PFS did not differ between the arms, even though a trend favouring bevacizumab over linifanib was observed. Also OS and ORR showed a similar trend. The most frequent AEs were neutropenia, thrombocytopenia, diarrhoea, fatigue, and palmar-plantar erytrodysestesia. Compared to bevacizumab arm, proteinuria and hypertension did not significantly differ, whereas a higher incidence of hypothyroidism and hyperbilirubinemia was reported. Patients receiving linifanib experienced more drug discontinuation compared to those bevacizumab treated. The main causes for discontinuation were fatigue, asthenia, left ventricular dysfunction, proteinuria and pulmonary embolism [51].

Linifanib was further tested in a phase II study with the goal of observing an ORR of at least 15% in 30 metastatic CRC patients progressing after standard chemotherapy. Unfortunately, the primary aim of the study was not reached as none of the 23 evaluable patients responded (ORR = 0%) (NCT01365910).

**5.1.5 MOTESANIB**

Motesanib (Amgen, Takeda) is an orally bioavailable, ATP competitive inhibitor of VEGFR family (VEGFR1-3) and KIT, and, with a lesser affinity, of PDGFR, RET, EGFR, Src and p38 kinase.

Motesanib inhibits VEGF-induced cellular proliferation and vascular permeability leading to tumor regression in vivo [52].

In a phase I open-label, dose-escalating study, motesanib administered at the MTD of 125 mg po qd showed activity in patients with advanced solid tumors (sarcoma, kidney, lung, colon, ovarian, thyroid and other), with a disease control rate of 56%, and stabilization for more than 6 months in 23% of the cases [53].

Motesanib demonstrated activity against metastatic medullary thyroid cancer, with 81% of disease stabilizations [54], and in GIST [55]. In advanced NSCLC patients, motesanib combined with carboplatin/paclitaxel resulted in modest clinical benefit in terms of PFR and ORR, and in increased toxicity leading to study discontinuation in patients with squamous histology [56].

As far as CRC is concerned, the safety and efficacy of motesanib was evaluated in association with chemotherapy (FOLFOX or FOLFIRI) with or without panitumumab in a phase 1b multicentre trial enrolling 119 metastatic patients. Panitumumab was early withdrawn from the study following toxicity reports in other trials. Overall, even though toxicity was manageable, clinical results were modest, with an ORR of 27% in first-line and 14% in second line setting [57].

The most common drug related adverse events were diarrhoea, nausea, anorexia, fatigue, and hypertension. Serious adverse events were common to other anti-VEGF agents and comprised thrombosis, pulmonary embolism and haemorrhage. Patients treated with motesanib experienced more frequently gallbladder related disorders, such as cholecystitis, cholelithiasis and gallbladder enlargement. Finally, thyroid disorders were reported (elevated serum thyroid stimulating hormone) [56,57].

### 5.1.6 TIVOZANIB

Tivozanib (Aveo) is an oral receptor TKI inhibiting VEGFR-1, -2, -3, and KIT and PGFRβ with less affinity [58]. In preclinical models tivozanib demonstrated in several human tumour xenografts, including colon cancer, to selectively block neo-angiogenesis reducing vascular permeability and decreasing microvessel density [59].

Tivozanib was compared to sorafenib in the TIVO-1, a phase III trial enrolling metastatic renal cancer patients, treatment naïve from prior anti VEGF and anti mTOR therapies [60]. Results were inconclusive as PFS was longer in tivozanib group and OS in sorafenib group.

In CRC setting, a phase Ib trial evaluated the safety, pharmacokinetics and antitumor activity of tivozanib combined with mFOLFOX6 in 30 metastatic patients. The MTD was defined at 1.5 mg po d1-14 q3w. The drug combination showed some activity as one patient had a clinical complete response, 10 had a partial response, and 11 obtained prolonged stable disease [61].

In a randomized phase II trial (BATON-CRC) previously untreated metastatic CRC patients received modified FOLFOX6 combined with tivozanib or bevacizumab and efficacy outcomes were similar in the two arms as median PFS (primary end point) was 9.4 months vs 10.7 months, and ORR was 45.2% vs 43.2% in tivozanib versus bevacizumab arm, respectively [62]. In this trial, neuropilin-1 levels were shown to be a potential biomarker for tivozanib activity as patients with low level of the protein presented a longer PFS [63]. Notwithstanding these data, a pivotal study for tivozanib in the treatment of neuropilin-1 (NPR-1) low CRC recently received negative feedback from FDA and the development of this anti VEGF agent is now on hold. Finally, in another phase II study the oral combination of tivozanib with everolimus was well tolerated with a 2-month PFS rate of 50% and a disease control rate of 50% [64].

The overall safety profile of tivozanib is comparable with the other VEGFR inhibitors, the most common adverse events being fatigue, diarrhoea, vomiting, neutropenia and hypertension [62,64].

**5.1.7 TREBANANIB**

Trebananib (Amgen) is an angiopoietin 1 and 2 (Ang-1 and Ang-2) neutralizing peptibody, with potential anti-angiogenic activity. It binds to Ang1 and Ang2, thereby preventing the interaction of the angiopoietins with their target Tie receptors [65]. Specifically, Ang-1 is a critical player in vessel maturation and it mediates migration, adhesion and survival of endothelial cells. Ang-2 disrupts the connections between the endothelium and perivascular cells and promotes cell death and vascular regression. This important role in the regulation of angiogenesis makes the

angiopoietin/Tie signaling pathway a therapeutically attractive target for the treatment of cancer [66]. In xenograft models dual inhibition of Ang1 and Ang2 provides greater suppression compared with the inhibition of single Ang1 or Ang2 [67].

Based on preclinical data, a phase I trial was conducted to evaluate the safety and antitumor activity of Trebananib in refractory advanced solid tumours. This study showed a manageable safety profile and evidence of efficacy as single agent, the greater tumour reduction being observed in an ovarian cancer case [68].

The results in colorectal cancer xenograft models and in the phase I study prompted researchers to evaluate the efficacy of trebananib in metastatic colorectal cancer patients in a phase II, double blind, placebo-controlled trial [69]. A total of 144 patients progressing during or within 6 months after the end of first-line oxaliplatin-based chemotherapy were randomized 2:1 to receive trebananib plus FOLFIRI versus placebo plus FOLFIRI. The study did not meet its primary endpoint, as trebananib did not prolong PFS compared to placebo (3.5 vs 5.2 months, p=0.33). Incidentally, 14% of patients included in the trebananib arm responded to therapy versus 0% in the placebo arm. The incidence of adverse events was similar in both study arms being the commonest diarrhoea, nausea and neutropenia. Incidence of grade 3 or greater adverse events was similar and included pulmonary embolism (1%), deep vein thrombosis (5%), and hypertension (1%).

More encouraging results were observed in ovarian and renal cancer. In the phase III TRINOVA-1 trial trebananib in combination with weekly paclitaxel prolonged significantly PFS in patients with recurrent ovarian cancer [70]. In metastatic renal cell cancer initial data indicate a superior efficacy when trebananib was added to sunitinib but also associated with a higher toxicity rate [71].

### 5.1.8 VANDETANIB

Vandetanib (CAPRELSA$^{TM}$, AstraZeneca) is another orally available receptor TKI that inhibits EGFR, VEGFR-2, RET, BRK, Tie2, members of the EPH receptor and Src kinase families in tumour cells and endothelial cells [72]. In vivo, vandetanib administration reduced tumor cell induced angiogenesis, tumour vessel permeability, and inhibited tumor growth and metastatic

spread in human xenograft models (breast, lung, prostate, colon, ovary, and vulva)[73]. In a phase I study conducted in refractory advanced tumours, vandetanib showed a manageable safety profile at the dose of 300 mg daily. No objective responses were reported, and the disease control rate ranged between 12,5% and 50% [74].

In CRC patients, vandetanib in combination with FOLFOX6 and FOLFIRI showed a manageable toxicity profile with no apparent pharmacokinetics interaction [75,76]. However, subsequent phase II randomized, placebo controlled studies did not demonstrate a meaningful clinical benefit from adding vandetanib to standard chemotherapy (NCT00454116, NCT00500292).

In a phase I trial vandetanib in combination with irinotecan and cetuximab was administered as second-line treatment, reporting an ORR of 7% and a disease control rate of 66% [77]. Toxicities were fairly manageable being grade 3 or 4 diarrhoea the most relevant toxicity (30%). However, while the primary endpoint of the study was safety, the observed efficacy raised concerns about moving forward with this combination. Finally, the combination of vandetanib with bevacizumab, oxaliplatin, and capecitabine resulted in unfavourable toxicity profile [78].

The most common adverse drug reactions (>20%) are diarrhoea/colitis, rash, acneiform dermatitis, hypertension, headache, nausea, upper respiratory tract infections, decrease appetite, and abdominal pain. Observed peculiar toxicities include prolonged QT interval, torsade de pointes, and sudden death. Thus, vandetanib is not indicated in patients suffering from congenital long QT syndrome and frequent monitoring of ECG and of serum potassium, calcium, magnesium, and TSH levels are recommended [79,80].

Vandetanib is licensed for symptomatic or progressive medullary thyroid cancer in patients with locally advanced or metastatic disease and in CRC setting further development is discontinued

### 5.1.9 VATALANIB

Vatalanib (Novartis) is an oral receptor TKI inhibiting VEGFR showing promising results in preclinical models and in patients with advanced solid tumor [81,82]. Vatalanib combined to FOLFOX4 revealed a well-tolerated toxicity profile and promising antitumor activity in metastatic

CRC patients [83]. Notwithstanding these encouraging preliminary results, vatalanib in association with oxaliplatin-based chemotherapy did not show to improve PFS in first line and OS in second-line setting in two phase III, randomized, placebo controlled trials conducted in CRC patients [84,85]. In the second line study a superior PFS value favouring vatalanib arm was reported (5.6 vs 4.2 months).

The most frequent reported adverse events include neutropenia, diarrhoea and hypertension. Grade 3 or greater adverse events are hypertension, diarrhoea, dizziness and pulmonary embolism [84,85]. Overall the efficacy data from the two abovementioned trials indicate a low activity of vatalanib in CRC patients [86] and this agent is not further investigated.

**5.2 Agents in early development**

**5.2.1 SEVACIZUMAB**

Sevacizumab (Jiangsu Simcere) is a humanized anti VEGF-A monoclonal antibody. Its maximal tolerated dose (MTD) is currently under investigation in a phase I study recruiting Chinese patients with advanced or metastatic solid tumors. This study is expected to complete enrolment at the end of 2015 (NCT01847118). A new phase Ib study exploring the safety profile of sevacizumab, its tolerability and pharmacokinetic parameters when combined to FOLFIRI in metastatic CRC patients who have failed first-line oxaliplatin based chemotherapy, has been already planned in China. The study is not yet open for recruitment (NCT02453464).

**5.2.3 SULFATINIB**

Sulfatinib (Hutchison Medi Pharma ) is an oral selective receptor TKI that targets VEGF and FGFR in early stage of development and phase I studies are currently evaluating its MDT, DLTs, pharmacokinetic parameters and preliminary antitumor activity in refractory advanced solid tumour. This drug has a manageable toxicity profile and early data indicate encouraging antitumor spectrum activity, with an ORR of 30% observed in HCC and NET patients [87].

**5.2.4 CABOZANTINIB**

*In vitro* biochemical and/or cellular assays have shown that cabozantinib (COMETRIQ$^{TM}$, Exelixis)

inhibits the tyrosine kinase activity of RET, MET, VEGFR-1, -2 and -3, c-KIT receptor, TRKB, AXL, and TIE-2 [88]. In addition to VEGFR inhibition, cabozantinib acts through several mechanisms of action that are supposed to enhance its antitumor activity. MET signalling pathway has been proposed to contribute to acquired resistance to antiangiogenic therapy and also to anti-EGFR therapy in colorectal cancer [88,89]. Moreover, MET receptor is a proto-oncogene and its activation promotes cell motility, proliferation, invasion and survival [90]. Cabozantinib is approved for the treatment of progressive, metastatic medullary thyroid cancer based on a double-blind, phase III trial comparing cabozantinib with placebo in 330 patients that showed a statistically significant improvement of PFS and response rates favouring Cabozantinib and a manageable toxicity profile [91].

In CRC cells cabozantinib has been evaluated in mouse xenograft models [92,93] and these preclinical data demonstrated significant tumour volume shrinkage with a reduction of intratumoral micro vessel density, suppression of the expression of VEGF-A in tumor tissues, and induction of apoptosis [92,93].

The clinical activity of cabozantinib in CRC patients is being evaluated in a phase I study conducted in the USA. This study is divided in three parts: 1) the combination dose finding cohort; 2) the combination expansion cohort; and 3) the monotherapy MET amplified cohort. In the first two cohorts, cabozantinib and panitumumab are administered in patients with KRAS wild-type metastatic CRC. In the monotherapy MET amplified cohort, fifty patients with RAS wild type tumors refractory to standard chemotherapy (including an anti-EGFR agent) will be screened for MET gene amplification and, if positive, will receive single agent cabozantinib. Primary aim of the study is the definition of the recommended phase II doses for the combination of cabozantinib and panitumumab and the objective response rate of single agent cabozantinib in patients with prospectively identified MET amplified metastatic CRC (NCT02008383).

Adverse reactions in patients treated with cabozantinib include diarrhoea, stomatitis, palmar-plantar erythrodysesthesia syndrome, decreased weight, decreased appetite, nausea, fatigue, oral pain, hair

colour changes, dysgeusia, hypertension, abdominal pain, and constipation. The most common laboratory abnormalities are elevation of liver function parameters, lymphopenia, hypocalcaemia, neutropenia, thrombocytopenia, hypophosphatemia, and hyperbilirubinemia. Grade 3-4 adverse reactions and laboratory abnormalities occurred in 5% of cabozantinib-treated patients. Fatal adverse reactions occurred in 6% of patients receiving cabozantinib and resulted from haemorrhage, pneumonia, septicaemia, fistulas, cardiac arrest, respiratory failure, and unspecified death [91].

### 5.2.5 TANIBIRUMAB

Tanibirumab (PharmAbcine) is a fully human monoclonal antibody that binds VEGFR-2. It showed antitumor activity against colorectal, breast, NSCLC, and glioblastoma xenografts and further studies demonstrated that it could positively synergize with other chemotherapy agents (CPT-11 or 5-FU) to inhibit tumour growth [94].

A phase I study of tanibirumab was conducted in patients with solid tumours refractory to standard chemotherapy to evaluate safety, pharmacokinetics, estimating maximum-tolerated dose (MTD) and recommended phase II dose. Treatment with tanibirumab showed tolerable toxicity profile and modest clinical efficacy (no objective response observed) [95].

### 5.2.6 VGX-100

VGX-100 (Circadian Technologies) is a novel fully human IgG1λ monoclonal antibody targeting VEGF-C, thus inhibiting activation of VEGFR-2 and VEGFR-3. In preclinical xenograft models, the combination of VGX-100 with bevacizumab and chemotherapy resulted in synergistic efficacy in several tumors [96].

VGX-100 safety profile, MTD and pharmacokinetic parameters were evaluated in a phase I study enrolling patients with advanced solid tumors refractory to standard chemotherapy. Patients included in the study, most of whom with colorectal cancer, were treated with VGF-100 as single agent or in combination with bevacizumab. MTD was not reached as toxicities were manageable up to the maximal pre-planned dose of 30 mg/kg qw. The most common grade 1-2 toxicities were

fatigue, rash, nausea, anorexia and hypertension. One patient experienced grade 3 hypertension and one patient a grade 3 worsening congestive heart failure. Antitumor activity of the combination of VGX-100 with bevacizumab was modest with a disease control rate of 12% [97].

### 5.2.7 APATINIB

Apatinib (AITAN$^{TM}$, LSK) is an orally bioavailable, receptor TKI that selectively inhibits VEGFR-2. Additionally it inhibits also RET, c-KIT and c-SRC and has some effect on EGFR, HER-2 and FGFR [98]. In preclinical models, apatinib was active against a broad spectrum of tumors [98].

A phase I study in patients with advanced solid tumours, demonstrated activity against gastrointestinal cancer, with a disease control rate of 83.8% [99]. Apatinib was especially investigated in advanced gastric cancer patients in phase II and III studies that confirmed the antitumor activity [100,101]. In a phase III study that recruited patients who failed at least two prior lines of therapy, apatinib compared to placebo demonstrated the prolongation of median OS and PFS [101].

The clinical activity and tolerability profile of apatinib in refractory metastatic CRC patients is being evaluated in a phase II, open label, randomized trial in which two doses of the drug (500 mg or 750 mg p.o, qd) are tested (NCT01531777). A total of 40 patients are planned to be enrolled and will continue therapy until disease progression, unacceptable toxicity or consent withdrawal. The study has recently been completed and results are waited.

Apatinib has a good safety profile [99-101], with grade 3-4 adverse reactions occurring in about 2% of the patients. The most frequent toxicities are hypertension, hand-foot syndrome, proteinuria, fatigue, anorexia and aminotransferase elevation [101].

### 5.2.8 VANUCIZUMAB

RO5520985 (RG7221, vanucizumab, Hoffmann-La Roche) is a bispecific human IgG1 antibody comprised of two different heavy chains and two different light chains. One arm of the antibody binds Angiopoietin-2 and the other binds VEGF-A. In preclinical models this antibody showed

potent tumour growth inhibition [102] and in a phase I study, it showed an acceptable toxicity profile at 30mg/Kg q2w, being hypertension, fatigue, headache and asthenia the most frequent adverse events [103].

Most recently, the results from a phase I study in patients with platinum-resistant/refractory epithelial ovarian cancer were presented at ASCO meeting 2015. Vanucizumab had an acceptable safety profile while demonstrating encouraging antitumor activity [104].

An ongoing phase II, multicentre, randomized, double blind trial is evaluating the efficacy and safety of RO5520985 plus mFOLFOX6 compared to bevacizumab plus mFOLFOX6 in untreated mCRC. This study will enrol 150 patients and it will be completed on May 2018 (NCT02141295).

**5.3 Compounds at a later stage of development**

**5.3.1 FAMITINIB**

Famitinib (Jiangsu HengRui Medicine) is a multi-target receptor TKI inhibitor structurally analogue to sunitinib with potent in vitro activity against c-KIT receptor, VEGFR-2 and 3, PDGFR, and RET [105].

The safety, pharmacokinetics and antitumor activity of famitinib in humans were evaluated in a phase I trial [106] that showed good tolerability and encouraging results in metastatic renal cancer, GIST and HCC. Phase II studies confirmed famitinib as an active and safe drug against metastatic renal cancer (disease control rate of 87% and median PFS of 10,7months) [107], and pre-treated metastatic HER2 negative breast cancer (ORR = 14,3%, PFS 58 days) [108].

A phase II study was performed in 154 patients with refractory metastatic CRC using a 2:1 randomization to receive famitinib or placebo. The study met its primary endpoint of a superior PFS in the famitinib group (2.8 vs 1.5 months, HR=0.58, p=0.0034). The ORR was 2.02% and 0.00% (p=0.54) and the disease control rate was 57.58% and 30.91% (p=0.0023), in the experimental and placebo group, respectively. The data of OS has not been presented, yet [109].

Famitinib has a good and manageable safety profile. In the phase I study the DLTs observed were grade 3 hypertension, hand foot skin reaction and diarrhoea [106]. In the phase II studies, the most

frequently reported adverse events included neutropenia, thrombocytopenia, hypertension, proteinuria, and hand-foot syndrome, all mostly of grade 1-2 [108,109].

### 5.3.2 FRUQUINTINIB

Fruquintinib (Hutchison Medi Pharma) is a novel receptor TKI inhibiting VEGFR-1, -2, -3. Its antitumor efficacy was demonstrated in multiple human tumour xenograft models [110]. Safety, pharmacokinetics and efficacy of two fruquintinib regimens were evaluated in a phase Ib trial enrolling CRC patients with metastatic disease refractory to previous therapies. The administration of 5 mg once daily for three weeks every four weeks showed a manageable toxicity profile and encouraging antitumor activity, with a disease control rate of 83.3% and a 16 week PFS of 65% [111].

Results of a randomized, double blind, placebo-controlled phase II trial evaluating fruquintinib vs placebo in the same patient setting were recently presented. The study met its primary endpoint as the median PFS was 4.7 months in the experimental arm versus 1.0 month in the placebo arm (HR =0.30, $p<0.001$), whereas disease control rate was 68.1% versus 20,8%, respectively [112].

Most common adverse events were hand-foot syndrome (61.7% of the patients), hypertension (51.4%), dysphonia (46.8%), proteinuria (44.7%) and AST elevation (27.7%) [112].

A phase III, randomized, double blind, placebo-controlled trial in refractory CRC patients is open for accrual in Chinese centres (NCT02314819). Primary endpoint of the study is overall survival and the study will be completed in late 2016.

### 5.3.3 NINTEDANIB

Nintedanib (VARGATEF$^{TM}$, BIBF 1120), an indolidone derivative, is an intracellular tyrosine kinase inhibitor, with activity against VEGFR family (VEGFR 1-3), FGFR 1-3 and PDGFRα and β. Nintedanib binds to the ATP binding pocket of the kinase domain, preventing the cross autophosphorilation of the receptor homodimers and stopping the signalling cascade. Inhibition of tumor growth was demonstrated in vivo in several xenograft models [113] and clinically in refractory tumors, demonstrating to be active in colorectal cancer, renal cancer and

hepatocarcinoma [114]. Nintedanib in combination with docetaxel is indicated in locally advanced or metastatic lung adenocarcinoma patients who failed a first-line treatment.

In CRC patients, the feasibility of weekly alternating sequence of Nintedanib and Afatinib in advanced tumors refractory to previous therapies was explored in a phase II trial. The disease control rate was 43.5%, with two patients who remained free from progression for more than 16 weeks [115].

A phase I/II open label randomized study of nintedanib plus mFOLFOX6 versus bevacizumab plus mFOLFOX6 was carried out in chemotherapy-naïve CRC patients. The primary endpoint of the study was not reached as the PFS rate at 9 months favoured bevacizumab arm. Noteworthy, ORR was superior in nintedanib arm (63,5% versus 56,1%) [116]. The role of nintedanib in pre-treated patients is currently investigated in the LUME-Colon 1 trial, a double bind, randomized phase III, placebo controlled study comparing nintedanib plus best supportive care vs placebo plus best supportive care. The end of the study is estimated on May 2016 (NCT02149108) [117].

Finally, Nintedanib is under development in hepatocarcinoma [118] and in breast, ovarian, oesophageal, endometrium, and thyroid cancer.

The most frequent adverse events of Nintedanib include diarrhoea, nausea, asthenia, neutropenia, vomiting, liver enzyme alteration, decrease appetite, hypertension, bleeding, gastrointestinal perforation, thrombotic events, venous and arterial thromboembolism [116,117].

## 6. CONCLUSION

Antiangiogenetic therapy targeting the VEGF system plays an important role in the management of mCRC, especially in improving OS. Since the approval of bevacizumab, aflibercept, regorafenib and very recently ramucirumab have been demonstrated to be active with a manageable toxicity profile and are now approved by regulatory Agencies. Consequently it is logical, then, that a relevant amount of new agents have been tested in the last years in the same disease setting. Most of them are orally available multiple receptor tyrosine kinase inhibitors with activity against multiple

tumours including thyroid cancer or renal cell cancer. Despite the relevant number of molecules tested, only famitinib, fruquintinib, and nintedanib advanced to a later stage of development in mCRC, whereas many others failed to demonstrate sufficient level of activity or to be superior to bevacizumab. The reasons why agents with similar mechanisms of action showed different efficacy results in the same patient setting are challenging and are worth of further consideration. Several factors are likely to play an important role in generating these contrasting results: the affinity of the drug for the target, the duration of signal inhibition (off-rate), the efficiency of drug penetration into tumor tissue, the pharmacodynamics and the pharmacokinetics of each single agents.

Newer agents are in a very early phase of development and phase I data showed promising preliminary results. Monoclonal antibodies against VEGF-C (VGX-100), VEGF-A and angiopoietin-2 (vanucizumab), and VEGFR-2 (tanibirumab) represent a new wave of innovation in this field.

## 7. EXPERT OPINION

Nowadays, antiangiogenic therapies represent a standard of care in mCRC patients, and several novel agents targeting the VEGF pathway have been tested in the last 10 years, mostly multi tyrosine kinase inhibitors. The multitarget-strategy action against tumour proliferation could explain why pharmaceutical companies identified this as a preferred area of research to develop new agents instead of drugs designed to inhibit one specific target. Unfortunately, several of these molecules did not demonstrate to be active in mCRC or were highly toxic and thus they have been abandoned. The severe toxicities observed, unknown drug antagonism, or issues related to treatment schedule and duration may explain at least in part the lack of positive results for many of the drugs tested. Importantly, however, many details of the mechanisms underlying neoangiogenesis, the relationship between growth factors involved in this process and tumor growth and metastatization, and the relationship between hypoxia and tumor cell metabolism and aggressiveness have not been completely described and understood, yet. Thus, a generic blockade of tyrosine kinases linked to

growth receptors may result in unexpected toxicity that may unacceptably counterbalance their potential activity.

On the other hand, the inhibition of a single target, like in the case of bevacizumab, might have a more "limited" activity, but allows a better definition of the role of each single pathway involved in the formation of new vessels, and a better exploration of the relative mechanisms of resistance. Compared to bevacizumab, aflibercept, binding either VEGFs and PlGF, and vanucizumab, binding either VEGF-A and Angiopoietin 2, are supposed to be more active as they circumvent two possible anti VEGF-A resistance mechanisms. As a result, new molecules, especially mAb with novel mechanisms of action, are emerging. Many of these drugs have been developed against targets potentially relevant in the mechanism of resistance to the anti-angiogenic strategy. Monoclonal Ab binding both VEGF-A and angiopoietin, or Abs binding VEGF-C or VEGFR-2 combined with chemotherapy resulted in encouraging results, that deserve validation in further trials. This study methodology, however, even though more "rationale" from a scientific point of view, could be less likely to result in immediate clinical benefits as it is for generic multi-target inhibition strategy, and this might not be preferred by pharmaceutical industries.

The definition of the best chemotherapy companion to anti-VEGF inhibition strategy is another interesting field of research. Multitarget TKIs are more active as single agent than in combination with chemotherapy, as demonstrated by regorafenib and by the emerging data for famitinib, fruquintinib, and nintedanib. This may be explained by the overlapping drug toxicity leading to frequent treatment delays or even discontinuation. Conversely, mAbs seemed to be more active when administered in combination with standard chemotherapy. Whether this should be irinotecan- or oxaliplatin-based remains to be explored.

Another unsolved issue and still unanswered concerns the identification of predictive markers able to identify a subgroup of patients who can potentially benefit more from anti-angiogenetic strategies. The definition of such predictive factors may potentially lead to a rescue of agents that showed marginal activity in a completely unselected patient population. This field of research is

likely to parallel biomolecular studies as the expression of particular proteins or growth factors may indicate main pathways involved in tumor cell growth, pointing out which specific mechanisms must be blocked in order to obtain the best antitumoral effects.

Finally, from a clinical point of view it should be defined which is the minimum clinically meaningful outcome gain of a new treatment. In fact, even though an experimental agent may statistically improve median overall survival of a specific patient population, it is to establish when this gain become clinically useful. As an example, it is questionable whether a 3-weeks gain in median overall survival is worth of potential toxicities which may impact on the quality of life. This was, for example, one of the most important concern of the CORRECT study [29], in which regorafenib versus placebo resulted in an advantage of 6 weeks gain in median overall survival versus placebo. This result permitted the registration of the drug by regulatory agencies in refractory metastatic CRC patients. Without questioning the role in clinical practice of already approved drugs, as for the development and approval of future drugs we agree with a large part of the scientific community asking for raising the bar in the definition of a clinically meaningful outcome: for instance, in the setting of pretreated patients,this could correspond to a threshold of at least 3-5 months of gain in median survival for the experimental arm versus best supportive care [119] Experimental agents that will not reach this threshold should be considered with interest from a scientific point of view, but with caution when considering their relevance in clinical practice.

In conclusion, we believe that a further understanding of the VEGF/VEGFR families and the mechanisms underlying neo-angiogenesis in experimental settings (both *in vitro* and *in vivo*) is mandatory. This could be clinically translated in a better patient selection, in order to maximize clinical impact and to the validation of new strategies against tumor proliferation.

**Declaration of Interest**

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***•A landmark study that put the basis for anti-angiogenic strategy in cancer therapies***

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Table 1. Novel anti-angiogenic compounds in CRC patients.

| **Compound** | **Company** | **Stage of development** | **Indication** | **Mechanism of action** | Primary endpoint of the study |
|---|---|---|---|---|---|
| | | | | | |
| **Inactive agents or with unfavourable safety profile not currently under development in CRC** | | | | | |
| Brivanib | Bristol-Myer Squibb | Phase III | Refractory mCRC | RTKI VEGFR-2, FGFR-1, -2 | OS |
| Cediranib | AstraZeneca | Phase III | First-line plus CT | RTKI VEGFR -1, -2, -3, PDGFRβ | PFS |
| Lenvatinib | Eisai | Phase I | | RTKI VEGFR -1, -2, -3, FGF-1, -2, -3, -4, PDGFRα, KIT, RET | MTD |
| Linifanib | Abbott | Phase II | Refractory mCRC | RTKI VEGFR -1, -2, -3, PDGFRβ | ORR |
| Motesanib | Amgen, Takeda | Phase Ib | First-second line mCRC | RTKI VEGFR-1, -2, -3, KIT, PDGFR, RET | ORR |
| Tivozanib | Aveo, Astellas | Phase II | Refractory mCRC | RTKI VEGFR-1, -2, -3, KIT, PDGFRβ | PFS |
| Trebananib | Amgen | Phase II | Second-line mCRC plus FOLFIRI | Ang-1, -2 inhibitor | PFS |
| Vandetanib | AstraZeneca | Phase II | Second line mCRC plus CT | RTKI EGFR, VEGFR-2, RET, BRK, TIE2 | Objective Disease Progression Events |
| Vatalanib | Novartis | Phase III | First line mCRC | RTKI VEGFR-1, -2 | PFS |
| | | | | | |
| **Agents in early development plan** | | | | | |
| Sevacizumab | Jiangsu Simcere | Phase Ib | Second-line plus FOLFIRI | mAb anti VEGF-A | MTD |
| Sulfatinib | Hutchison Medi Pharma | Phase I | Solid Tumors | RTKI VEGFR, FGFR | MTD |
| Cabozantinib | Exelixis | Phase I | RAS wt, refractory mCRC | RTKI VEGFR-1, -2, -3, RET, MET, KIT, | MTD |

| | | | | | |
|---|---|---|---|---|---|
| | | | | TRKB, AXL, TIE-2 | |
| Tanibirumab | PharmAbcine | Phase I | Solid Tumors | mAb anti VEGFR-2 | MTD |
| VGX-100 | Circadian Technologies | Phase I | Refractory mCRC | mAb Anti VEGF-C | MTD |
| Apatinib | LSK | Phase II | Refractory mCRC | RTKI VEGFR-2, RET, KIT, c-SRC | ORR |
| Vanucizumab | Roche | Phase II | First-line plus mFOLFOX6 | mAb anti VEGF-A, Ang-2 | PFS |
| | | | | | |
| **Compounds at a later stage of development** | | | | | |
| Famitinib | Jiangsu HengRui Medicine | Phase II | Refractory mCRC | RTKI VEGFR-2, -3, KIT, PDGFR, RET | PFS |
| Fruquintinib | Hutchison Medi Pharma | Phase III | Refractory mCRC | RTKI VEGFR-1, -2, -3 | OS |
| Nintedanib | Boehringer Ingelheim, | Phase III | Refractory mCRC | RTKI VEGFR-1, -2, -3, FGFR, PDGFR α/β | OS |
| | | | | | |

mCRC = metastatic colorectal cancer; RTKI = receptor tyrosine kinase inhibitor; CT = Chemotherapy; Ang-1 -2 = Angiopoietin -1 -2; mAb = monoclonal Antibody; mFOLFOX6 = modified FOLFOX6 (5Fluorouracil, leucovorin, oxaliplatin); FOLFIRI = 5Fluorouracil, leucovorin, irinotecan; RAS = All RAS (Rat Sarcoma viral oncogene homolog); OS = Overall Survival; PFS = Progression Free Survival; MTD = Maximal Tolerated Dose; ORR = Overall Response Rate

Figure 1. VEGF pathway and its inhibitors (Adapted with permission from [4] with permission of Taylor & Francis).